Esp

Week 4

Summarized below are the experiments conducted this week in chronological order. Click on the experiment name to view it. To go back to this summary, click **Summary** in the footer.

Summary

1 PCR amplification and purification of pSB1K3-T7-EB and pSB1K3-T7-EC 2

2 Expression of pSC1K3-T7-EB and pSC1K3-T7-EC by IPTG-induction 5

1 PCR amplification and purification of pSB1K3-T7-EB and pSB1K3-T7-EC

Responsible

Oskar Ohman

Protocol Used

Colony Picking
PCR Amplification
PCR Purification

Experimental Set Up

Description: 6 colonies containing the ligation product pSB1K3-T7-EB were picked from two different plates, 3 colonies from each plate. 3 colonies containing the ligation product pSC1K3-T7-EC were picked from another plate. These samples are hereafter referred as:

Plate 1: EB5.1.1, EB5.1.2, EB5.1.3
Plate 2: EB5.2.1, EB5.2.2, EB5.2.3
Plate 3: EC4.1, EC4.2, EC4.3

For 9 PCR reactions a mixture with the following composition was made:

Table 1: Set up for 9 PCR reactions.

Component	**Volume (μl)**
2x PCR Master Mix Q5 Polymerase	112.5
VF_2F	27
VF_2R	27
dH2O	58.5

25 μl of this mixture was mixed with each colony in a PCR tube. After the PCR amplification gel electrophoresis was run at 100 V, 30 min.

The concentration of the samples were measured with NanoDrop.

Modifications and comments to protocols

Sample EB5.1.1, EC4.2 and EC4.1 were purified. EB5.1.1 and EC4.2 were eluted with 50 μl elution buffer and re-eluted in 30 μl elution buffer. EC4.1 was eluted in 25 μl elution buffer.

Results

The gel showed bands in the area 1200 bp, where the constructs pSB1K3-T7-EB and pSB1K3-T7-EC were expected to be. However, the gel looked smeary in all wells except well 4. In well 4 an unwanted band in the area 800 bp was detected.

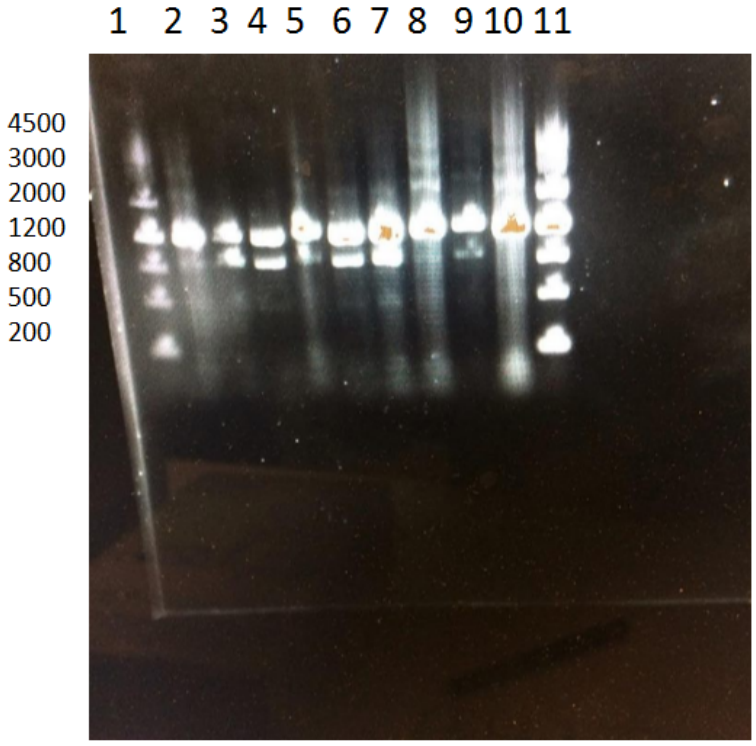

Figure 1: Results from gel electrophoresis after Colony PCR amplification.

Table 2: The order of the wells

Well	Sample
1	Ladder
2	EB5.1.1
3	EB5.1.2
4	EB5.1.3
5	EB5.2.1
6	EB5.2.2
7	EB5.2.3
8	EC4.1
9	EC4.2
10	EC4.3
11	Ladder

Discussion and Troubleshooting

The unwanted bands in the gel could be due to contamination. The concentration of sample EB5.1.1, EC4.1 and EC4.2 were considered high enough to be send for sequencing. As seen in Results, only concentrations high enough for sequencing were documented.

2 Expression of pSC1K3-T7-EB and pSC1K3-T7-EC by IPTG-induction

Responsible

Sigrun Stulz, Oskar Ohman

Protocol used

Colony Picking
Protein Expression

Experimental Set up

Description: The aim of this experiment was to express the proteins expressed by pSB1K3-T7-EB and pSB1K3-T7-EC by IPTG induction. 7 colonies were picked from plate EB4.1 with BL21 cells containing pSC1K3-T7-EB and 7 colonies from plate EC with BL21 cells containing pSC1K3-T7-EC. The colonies were incubated in liquid medium for 37°C. When the OD reached 0.6 the protein expression was induced with 0.5 M IPTG of different final concentrations and with different incubation times as can be seen in Table 3.

Table 3: 14 colonies were incubated at 37°C for 3 or 4 hours, whereof 12 colonies were IPTG induced with different IPTG concentrations .

Plate name	Final IPTG concentration (mM)	Incubation time, 37 C (h)
EB4.1	0	4
EB4.1	0.1	3
EB4.1	0.5	3
EB4.1	1	3
EB4.1	0.5	4
EB4.1	0.5	4
EB4.1	1	4
EC4	0	4
EC4	0.1	3
EC4	0.5	3
EC4	1	3
EC4	0.1	4
EC4	0.5	4
EC4	1	4

Results

SDS-PAGE results for this experiment can be seen in ESP Week 5.

Discussion and Troubleshooting

By mistake a duplicate was made for sample EB4.1 0.5 mM IPTG concentration, 4 h, because of unclear labelling. Therefore no sample with a colony from EB4.1, 0.1 mM IPTG concentration, 4h, was made.